15 GABAPENTIN QUANTITATION AND CONFIRMATION BY LCMS

15.1 Summary

15.1.1 Gabapentin is extracted from biological samples with an acetonitrile precipitation and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

15.2 Specimen Requirements

15.2.1 One mL blood, biological fluid or tissue homogenate.

15.3 Reagents and Standards

15.3.1 Ammonium acetate

15.3.2 Methanol

15.3.3 Acetonitrile

15.3.4 Gabapentin (Neurontin® from Parke-Davis)

15.3.5 Phenacetin (Merck)

15.4 Solutions, Internal Standards, Calibrators and Controls

15.4.1 10 mM Ammonium acetate: Weigh 0.38 g ammonium acetate. Transfer to 500 mL volumetric flask and QS to volume with dH_2O

15.4.2 Working standard solutions for gabapentin

15.4.2.1 10 mg/mL gabapentin stock solution. Weigh 100 mg gabapentin, transfer to 10 mL volumetric flask and QS to volume with dH_2O.

15.4.2.2 0.1 mg/mL gabapentin working solution: Pipet 100 µl of 10 mg/mL stock solution of gabapentin into 10 mL volumetric flask and QS to volume with dH_2O

15.4.3 Quality Control (QC) standard solutions

15.4.3.1 0.1 mg/mL gabapentin QC solution: Pipet 100 µl of separate 10 mg/mL stock solution of gabapentin into 10 mL volumetric flask and QS to volume with dH_2O

15.4.4 Internal standard working solution

15.4.4.1 1 mg/mL phenacetin stock solution. Weigh 10 mg phenacetin, transfer to 10 mL volumetric flask and QS to volume with methanol.

15.4.4.2 0.1 mg/mL phenacetin: Pipet 1 mL of 1 mg/mL phenacetin stock solution into 10 mL volumetric flask and QS to volume with dH_2O

15.4.5 Calibrators. To prepare the calibration curve, pipet the following volumes of working solution into appropriately labeled 16 x 125 mm screw cap tubes:

15.4.5.1 Cal 1: 50 mg/L gabapentin: 500 µL of 0.1 mg/mL gabapentin working solution

15.4.5.2 Cal 2: 20 mg/L gabapentin: 200 µL of 0.1 mg/mL gabapentin working solution

15.4.5.3 Cal 3: 10 mg/L gabapentin: 100 µL of 0.1 mg/mL gabapentin working solution

15.4.5.4 Cal 4: 5 mg/L gabapentin: 50 µL of 0.1 mg/mL gabapentin working solution

15.4.5.5 Cal 5: 2 mg/L gabapentin: 20 µL of 0.1 mg/mL gabapentin working solution

15.4.5.6 Cal 6: 1 mg/L gabapentin: 10 µL of 0.1 mg/mL gabapentin working solution

15.4.5.7 For each calibrator, add 1 mL blank blood to each tube to achieve final concentration.

15.4.6 Gabapentin Control (QC)

15.4.6.1 15 mg/L gabapentin: Pipet 150 µL of 0.1 mg/mL gabapentin QC Solution into appropriately labeled 16 x 125 mm screw cap tube and add 1 mL blank blood.

15.4.6.2 Negative blood control: Blood bank blood (or equivalent) previously determined not to contain gabapentin.

15.5 Apparatus

15.5.1 Screw cap test tubes, 16 x 125 mm

15.5.2 Screw cap test tubes, glass, conical bottom

15.5.3 Centrifuge capable of 2,000-3,000 rpm

15.5.4 Nitrogen evaporator with heating block

15.5.5 Vortex mixer

15.5.6 GC autosampler vials with inserts

15.5.7 LC/MS: Agilent Model 1100 LC-MSD

15.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified or adjusted to improve separation and sensitivity.

15.5.7.1.1 Elution conditions:

15.5.7.1.1.1 Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 µm particle size
15.5.7.1.1.2 Column thermostat: 30° C
15.5.7.1.1.3 Solvent A: 10 mM ammonium acetate in dH_2O
15.5.7.1.1.4 Solvent B: methanol
15.5.7.1.1.5 Isocratic elution, stop time: 6.00 min

Time	Solv. B	Flow
0.00	40	0.5

15.5.7.1.2 Spray Chamber

15.5.7.1.2.1 Ionization Mode: Electrospray
15.5.7.1.2.2 Gas Temperature:350° C
15.5.7.1.2.3 Drying Gas (N_2): 12.0 l/min
15.5.7.1.2.4 Nebulizer pressure: 30 psig
15.5.7.1.2.5 Vcap (Positive): 4000 V

15.5.7.1.3 Selected Ion Monitoring

15.5.7.1.3.1 Polarity: Positive
15.5.7.1.3.2 SIM parameters (quantitation ion)

Time	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0	gabapentin	95.0	170	1.0	Low	218
		137.0	170			218
		154.0	170			218
		172.0	170			218
3.0	Phenacetin	110.0	170	1.0	Low	292
		138.0	170			292
		180.0	170			292

15.6 Procedure

15.6.1 Label 16 x 125 mm screw cap tubes appropriately with blank, calibrators, controls and case sample IDs.

15.6.2 Prepare calibrators and controls.

15.6.3 Add 1 mL case specimens to the appropriately labeled tubes.

15.6.4 Add 50 µL 0.1 mg/mL phenacetin internal standard working solution to each tube.

15.6.5 Slowly, add dropwise 2 mL cold (freezer temperature) acetonitrile to each tube while vortexing. Continous vortexing, not mere mixing, is essential.

15.6.6 Vortex an additional 30 seconds.

15.6.7 Place tubes in freezer for at least 30 minutes to facilitate separation.

15.6.8 Centrifuge at approximately 2500 rpm for 15 minutes.

15.6.9 Transfer top (acetonitrile) layer to clean conical bottom tubes taking care not to transfer any lower layers.

15.6.10 Evaporate to dryness at approximately 50° C under nitrogen.

15.6.11 Reconstitute samples in 100 µL methanol. Vortex briefly. Transfer to GC autosampler vials.

15.6.12 Inject 1 µL of each sample on LC/MS in the API-ES/SIM Mode

15.7 Calculation

15.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.

15.8 Quality Control

15.8.1 See Toxicology Quality Guidelines

15.9 References

15.9.1 C MacDonald and P Zavitsanos. Determination of Gabapentin in Human Serum by LC/MS. Agilent Application Note.

15.9.2 D Ifa, M Falci, M Moraes, F Bezerra and G deNucci. Gabapentin Quantification in Human Plasma by LC-ESI-MS. *J Mass Spec* 36: 188-194, 2001.

15.9.3 C Wolf, J Saady and A Poklis. Determination of Gabapentin in Serum using Solid Phase Extraction and GC/MS. *J Anal Tox* 20:498-501, 1996.

15.9.4 J Pearson and R Steiner, in-house development.